Persistence of Both Peripheral and Non-Peripheral Corneodesmosomes in the Upper Stratum Corneum of Winter Xerosis Skin *Versus* only Peripheral in Normal Skin

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To understand the biochemical abnormalities that underlie the reduced desquamation observed in dry skin, we analyzed corneodesmosome degradation in normal and winter xerosis skin. Western blotting of total proteins from corneocytes obtained by varnish-strippings from the legs of 56 volunteers with normal (26) or xerotic (30) skin was performed using antibodies specific for (corneo)desmosome proteins. In the whole population, the amounts of desmoglein 1 and plakoglobin were found to be correlated, but were not related to the amounts of corneodesmosin. This suggests simultaneous proteolysis for the former proteins differing from that of corneodesmosin. Neither entire desmoplakins nor any proteolysis-derived fragments were detected. The amounts of corneodesmosin, desmoglein 1, and plakoglobin detected were found to be significantly higher in xerotic compared with normal skin extracts. Conventional and freeze-fracture electron microscopy showed the absence of nonperipheral corneodesmosomes in the upper stratum corneum of normal skin but the presence of a significant number of these structures in the same layer of winter xerosis skin. These results provide a more precise description of the proteolysis of corneodesmosome components in the upper cornified layer of the epidermis. They support previous studies demonstrating the importance of corneodesmosome degradation in desquamation and reveal that the nonperipheral corneodesmosomes, which are totally degraded during maturation of the stratum corneum in normal skin, persist in winter xerosis, probably leading to abnormal desquamation. ***Key words: desmosomes/epidermis/keratinocytes/proteases. J Invest Dermatol 116:23–30, 2001***

The main function of the stratum corneum (SC) is to protect the organism from dehydration and exposure to physical, chemical, or biologic insults from the environment. In the last 20 y, considerable efforts have been made to describe the structures formed during the last steps of keratinocyte differentiation, and to identify the molecular components and function of corneocytes (for reviews see Reichert *et al*, 1993; Holbrook, 1994; Simon, 1994; Roop, 1995).

Beside intercellular lipids, which are thought to play a major role in preventing water loss (Elias and Menon, 1991; Williams, 1991; Rawlings *et al*, 1994a; Schaefer and Redelmeier, 1996), particular attention has been given to SC protein structures derived from desmosomes and called corneosomes or corneodesmosomes (Chapman and Walsh, 1990; Serre *et al*, 1991). Desmosomes undergo important morphologic modifications when the keratinocytes reach the cornified layers: the desmosomal plaque is no longer visible, and, in the extracellular space, a homogeneous electron-dense plug is observed instead of the characteristic symmetrical tri-lamellar structure of the desmosomal core (Menton and Eisen, 1971a; Allen and Potten, 1975). Corneodesmosomes play a major role in SC cohesion (King *et al*, 1979; Chapman *et al*, 1991). In nonpalmo-plantar skin a large majority of corneodesmosomes are degraded in the lower SC; however, those associated with the corneocyte interdigitations at the periphery of the cells persist up to the surface of the skin (Skerrow *et al*, 1989; Chapman and Walsh, 1990; Serre *et al*, 1991; Haftek *et al*, 1998). For desquamation to occur, all persistent peripheral corneodesmosomes must be degraded at the skin surface (Lundström and Egelrud, 1988; Egelrud and Lundström, 1990). Corneodesmosome degradation probably involves the SC chymotryptic enzyme (Egelrud and Lundström, 1991; Egelrud, 1993; Hansson *et al*, 1994; Sondell *et al*, 1995) and other less well-known proteases such as trypsin-like proteases (Suzuki *et al*, 1993, 1994, 1996). Although corneodesmosomal degradation is an important part of desquamation, how this necessarily tightly ordered process is regulated remains to be elucidated.

Corneodesmosomes have been described for many years; however, the biochemical modifications associated with their formation remain unclear, mainly because these structures are partly incorporated into the cornified cell envelopes (Haftek *et al*, 1991; Serre *et al*, 1991) and therefore cannot be purified. Desmoglein 1

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Abbreviations: Cdsn, corneodesmosin; Dsc, desmocollin; Dsg, desmoglein; MoAb, monoclonal antibody; SC, stratum corneum.

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(Dsg1) and desmocollin 1 (Dsc1) are thought to be the two major corneodesmosomal components responsible for intercorneocyte cohesion (Egelrud and Lundström, 1989; Lundström and Egelrud, 1990; Arnemann *et al*, 1993).[1] Dsgs and Dscs are calcium-dependent desmosomal glycoproteins of the cadherin family. Both are known to exist as three different isoforms encoded by three different genes (e.g., DSG1–3 for Dsgs). Their expression is tissue specific and differentiation dependent. In the epidermis, Dsg2 is expressed in the basal layer whereas Dsc2 is expressed throughout the living layers, Dsg3 and Dsc3 are found in the basal and spinous layers, and Dsg1 and Dsc1 are expressed in the uppermost layers. The desmosomal cadherins are functionally associated to the cytoskeleton intermediate filaments via cytoplasmic desmosomal plaque proteins including plakoglobin (also known as γ-catenin), desmoplakins, and plakophilins (for reviews see Kowalczyk and Green, 1996; Garrod *et al*, 1996). We identified an epidermal protein, referred to as corneodesmosin (Cdsn), and demonstrated its involvement in corneodesmosome functions (Serre *et al*, 1991; Haftek *et al*, 1997; Simon *et al*, 1997; Guerrin *et al*, 1998). Synthesized in the upper spinous and granular layer keratinocytes as a 52–56 kDa glycosylated and phosphorylated protein, Cdsn is secreted via lamellar bodies. It is associated to the desmosomal core just before the transformation of desmosomes into corneodesmosomes. In the cornified layers, corneodesmosome plugs were also shown to contain the protein. During SC maturation, Cdsn is progressively proteolyzed. In the upper SC, the 52–56 kDa form is no longer detected and 36–30 kDa fragments of the protein become predominant. Further degradation of the smaller fragments, at the skin surface, results in the cleavage of two serine-rich amino- and carboxy-terminal domains of Cdsn, believed to be responsible for the adhesion properties of the protein. A close association also exists between complete Cdsn degradation and corneocyte shedding in an *in vitro* model of desquamation (Lundström *et al*, 1994). As the location, the biochemical characteristics, the processing stages, and the amino acid sequence of Cdsn are similar in several mammals (Montézin *et al*, 1997; Velten *et al*, 1998), the protein is probably essential for the function of corneodesmosomes, and thus for corneocyte cohesion.

Numerous ichthyoses and xeroses are characterized by skin dryness and by an accumulation of scales (Menton and Eisen, 1971b; Ghadially *et al*, 1992; Menon *et al*, 1992; Vicanova *et al*, 1996). It appears that reduced degradation of nonperipheral corneodesmosomes is associated with ichthyoses (Ghadially *et al*, 1992; Fartasch, 1997; Haftek *et al*, 1997). In soap-induced xerosis, nonperipheral corneodesmosomes also remain undegraded in the upper SC (Rawlings *et al*, 1994b).[1]

Here, we compared the degradation of corneodesmosomes in the SC of normal and winter xerosis skin. Our results demonstrated increased amounts of corneodesmosomal proteins, and persistence of nonperipheral corneodesmosomes, in xerotic skin. We speculate that these facts might be the cause of the hyperkeratosis and abnormal desquamation found in winter xerosis.

MATERIALS AND METHODS

Subjects Fifty-six female Caucasians with normal to dry skin, aged 22–49 y, and without any history of genetic skin disorders or atopy, participated in this study. The skin dryness of the external part of their legs was evaluated, using clinical criteria, at the end of the winter by two experts including a dermatologist, and was graded as follows: 26 individuals with normal skin and 30 individuals with moderate to well-defined winter xerosis, i.e., dry skin characterized by roughness and papyraceous appearance of the skin, presence of raised squames and/or scales, and irritation.

[1]Long S, Banks J, Watkinson A, *et al*: Desmocollin 1: a key marker for desmosome processing in the stratum corneum. *J Invest Dermatol* 106:872, 1996 (abstr.)

Monoclonal antibodies and western blotting The anti-Cdsn monoclonal antibodies (MoAb) G36-19 and F28-27 are part of a series of antibodies produced and previously characterized in our laboratory (Serre *et al*, 1991; Simon *et al*, 1997). DG3.10, a MoAb directed to Dsg1 and Dsg2, PG5.1, a MoAb directed to plakoglobin, and a cocktail of DP1 and 2-2.15, DP1 and 2-2.17, and DP1 and 2-2.20, three MoAb directed to desmoplakins 1 and 2, were purchased from Progen Biotechnik (Heidelberg, Germany). An anti-γ-catenin MoAb from Transduction Laboratories (Lexington, KY) was also used. The anti-γ-catenin and the G36-19 and F28-27 MoAb were diluted to 0.1 μg per ml, DG3.10 and PG5.1 to 0.5 μg per ml, and the antidesmoplakin cocktail to 1:1000. The western blotting method has been described in detail previously (Montézin *et al*, 1997). In particular, peroxidase-conjugated sheep immunoglobulins to mouse IgG (Biosys, Compiègne, France) were revealed with the ECL western blotting kit (Amersham, Aylesbury, U.K.).

Analysis of proteins extracted from human epidermis and superficial SC As described previously (Simon *et al*, 1997), dermo-epidermal cleavage of breast skin (obtained from patients undergoing plastic surgery) was performed by heat treatment, and the epidermis was sequentially homogenized in the presence of detergent and then with 8 M urea.

Superficial SC extracts were obtained from the volunteers by three consecutive varnish-strippings, essentially as described previously (Guerrin *et al*, 1998). The samples were recovered by filtration, washed four times in acetone, and air dried. The resulting powdery material was boiled for 10 min in 62.5 mM Tris-HCl, pH 6.8, containing 2% sodium dodecyl sulfate and 0.2 M dithiothreitol, and the suspensions were clarified by centrifugation ($10{,}000 \times g$, 10 min).

Protein concentrations in the extracts were evaluated using the Protein Assay (BioRad Laboratories, Richmond, CA). Laemmli's sample buffer was added to the extracts to adjust the concentrations, and the proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), stained by Coomassie blue, or analyzed by western blotting. The immunoblotting reactivities, related to the detectable amounts of proteins, were scanned and quantified by densitometry using the Intelligent Quantifier 1-D software (Bio Image, Ann Arbor, MI).

Freeze-fracture electron microscopy The procedure was applied to whole SC freshly obtained varnish-strippings from ten selected subjects, three with a normal skin and seven affected by a severe winter xerosis. After rinsing at room temperature in 0.1 M phosphate-buffered saline, pH 7.2, the strippings were cut into 2 mm × 3 mm rectangles and placed between two standard copper plates (Craane-Van Hinsberg *et al*, 1997). The specimen sandwiches were quenched by jet freezing with high-speed liquid propane and inserted into the spring-loaded double replica device, which was submerged in liquid nitrogen. Fracturing was performed at −115°C in a vacuum of 2×10^{-7} torr in a freeze-fracture unit (BAF 400 T, Balzers, Liechtenstein): releasing the closing spring opened the double replica device and separated the two copper plates. The samples were fractured mainly parallel to the anatomic surface of the SC, and the fracture planes were replicated. Platinum/carbon replicas were floated on 14% sodium hypochlorite, rinsed in double-distilled water, collected using 400 mesh copper grids, immersed in chloroform-methanol (2:1, vol/vol), and finally air dried. They were examined in a Zeiss CEM 902 transmission electron microscope. Corneodesmosome density (corneodesmosome area divided by total area) was measured on random micrographs (7 cm × 7 cm) from two normal and three xerotic samples.

Freeze-fracture immunocytochemistry The procedure was essentially performed as described by Torrisi and Mancini (1996). Small pieces (2 mm × 3 mm) of varnish-strippings from two subjects with xerosis skin were fixed for 1 h at 4°C in 0.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer, pH 7.4. The samples were impregnated gradually in 30% glycerol, fast-frozen in liquid ethane maintained at −75°C, and crushed under liquid nitrogen. Fractured samples were thawed in a buffered solution of 30% glycerol containing 0.5% glutaraldehyde, deglycerinated in 1 mM glycyl-glycine (Sigma Chemical) and thoroughly washed before immunochemical labeling. After preincubation with 2.5% fetal bovine serum for 30 min, freeze-fractured samples were incubated with anti-Cdsn MoAb for 1 h. After washing, they were incubated overnight at 4°C with goat antimouse IgG conjugated to 10 nm gold particles (British BioCell International, Cardiff, U.K.). Negative controls were performed omitting the primary antibody. After rinsing, the samples were osmicated, block-stained with uranyl acetate, and dehydrated in ethanol. Fracture-labeled samples were embedded in Epon 812, and thin sections, mounted on Formvar-coated

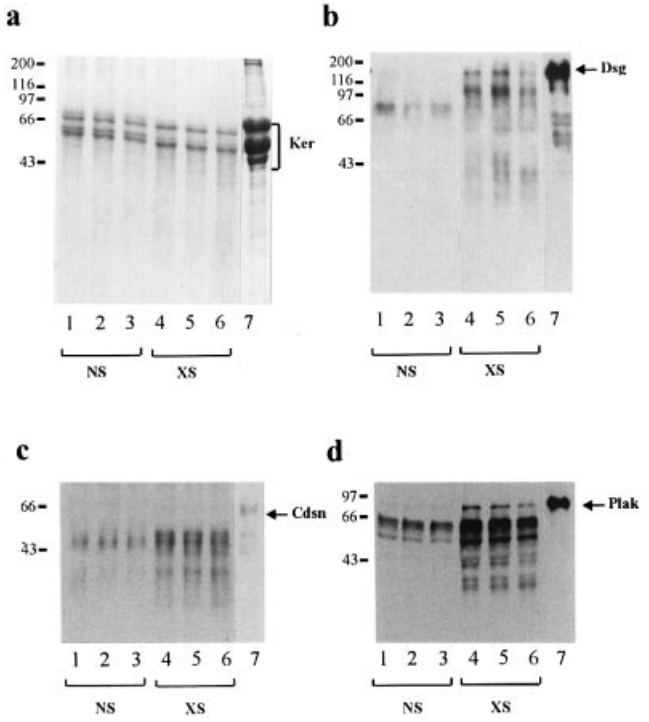

Figure 1. Various amounts of corneodesmosomal proteins are observed in upper SC extracts. Equal amounts of total proteins corresponding to three serial strippings performed on one individual with normal skin (NS, *lanes 1–3*) and on one individual with xerotic skin (XS, *lanes 4–6*), and, as a control, proteins of a Tris-urea buffer extract of breast epidermis (*lane 7*) were analyzed. The proteins were separated by SDS-PAGE, stained with Coomassie blue (*a*), or transferred to nitrocellulose membranes and immunoblotted with (*b*) DG3.10, a MoAb directed to Dsg1 and Dsg2, (*c*) G36-19, a MoAb directed to Cdsn, and (*d*) PG5.1, a MoAb directed to plakoglobin. The position of molecular mass standards (kDa) is indicated on the left. The arrows show entire 160 kDa Dsg1 (*b*), the 52–56 kDa Cdsn (*c*), and entire 83 kDa plakoglobin (Plak, *d*).

grids, were examined in a transmission electron microscope. Alternatively, to obtain fracture-labeled replicas, the fracture-labeled samples were dried using a sublimation dehydrant, Peldri II, mounted on adhesive tapes, and replicated by platinum/carbon evaporation in the Balzers freeze-fracture unit at ambient temperature in a vacuum of 2×10^{-6} torr. The replicas were immersed in sodium hypochlorite, mounted, and observed as described above.

Conventional transmission electron microscopy Small pieces of varnish-stripping were fixed for 2 h at 4°C with 2% acrolein in 0.1 M sodium cacodylate buffer, pH 6.8, and postfixed at room temperature in a mixture of 0.25% ruthenium tetroxide and 0.5% potassium ferrocyanide in the same buffer. After washing, samples were dehydrated and embedded in Epon 812. Thin sections were examined after double staining with uranyl acetate and lead citrate.

Statistical analysis Statistical significance of the differences in corneodesmosome proteins was evaluated using the Mann and Whitney *U* test. Mean values of corneodesmosome density were compared using Student's *t* test.

RESULTS

Increased amounts of corneodesmosomal proteins are detected in the upper SC of winter xerosis skin Superficial SC extracts were obtained from 56 volunteers with normal ($n = 26$) or dry ($n = 30$) skin by three sequential varnish-strippings. The dry weight of material removed from normal and winter xerosis skin was measured and found to be nonsignificantly different in the two groups (103 ± 41 μg per cm^2 *vs* 137 ± 46 μg per cm^2 for the outer strippings; 272 ± 65 μg per cm^2 *vs* 372 ± 106 μg per cm^2 for the cumulative amounts corresponding to the three sequential strippings). Identical results were obtained when the total amount of proteins extracted was computed (not shown). The protein concentration was adjusted to 1 mg per ml and the same amount of material for each extract was separated by gel electrophoresis, as confirmed by Coomassie blue staining (**Fig 1*a***).

When the extracts were analyzed by western blotting with DG3.10, the antibody specific for the ~90 kDa cytoplasmic domain of Dsg1 and Dsg2 stained three bands of 160, 95, and 80 kDa

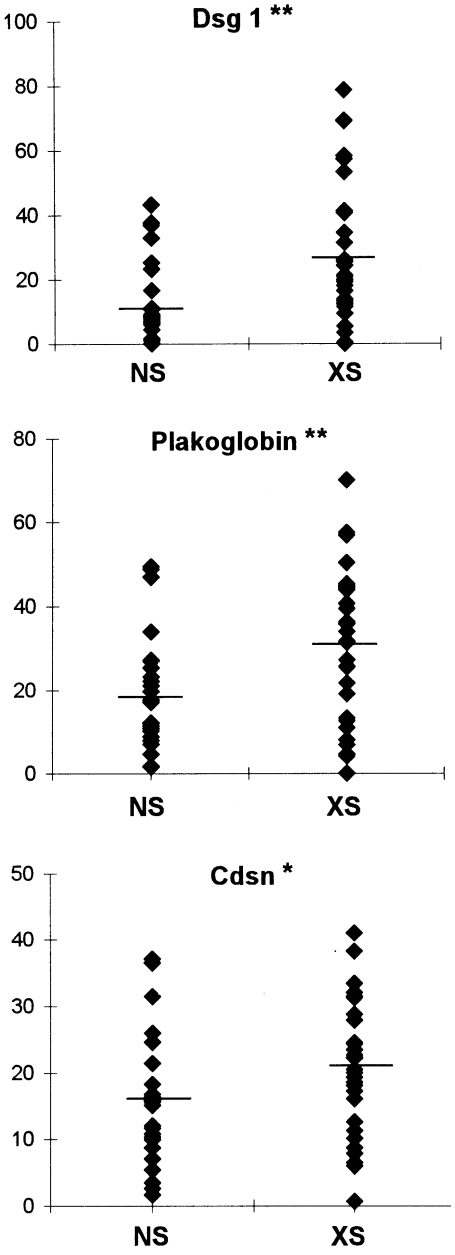

Figure 2. The amounts of Dsg1, plakoglobin, and Cdsn are increased in the SC extracts of individuals with winter xerosis skin. Equal amounts of total proteins corresponding to the first stripping performed on the 56 individuals were analyzed by western blotting with DG3.10, a MoAb directed to Dsg1, with a mix of PG5.1 and an anti-γ-catenin antibody, two MoAb directed to plakoglobin, and with a mix of G36-19 and F28-27, two MoAb directed to Cdsn. The immunoreactive bands were then quantified by densitometry. The values obtained for the 26 individuals with normal skin (NS) and the 30 individuals with xerotic skin (XS) are represented by squares, on an arbitrary scale. The horizontal lines correspond to the calculated medians. Note the significantly increased amounts of Dsg1, plakoglobin, and Cdsn in xerotic skin compared with normal skin (*$p = 0.05$; **$p < 0.02$).

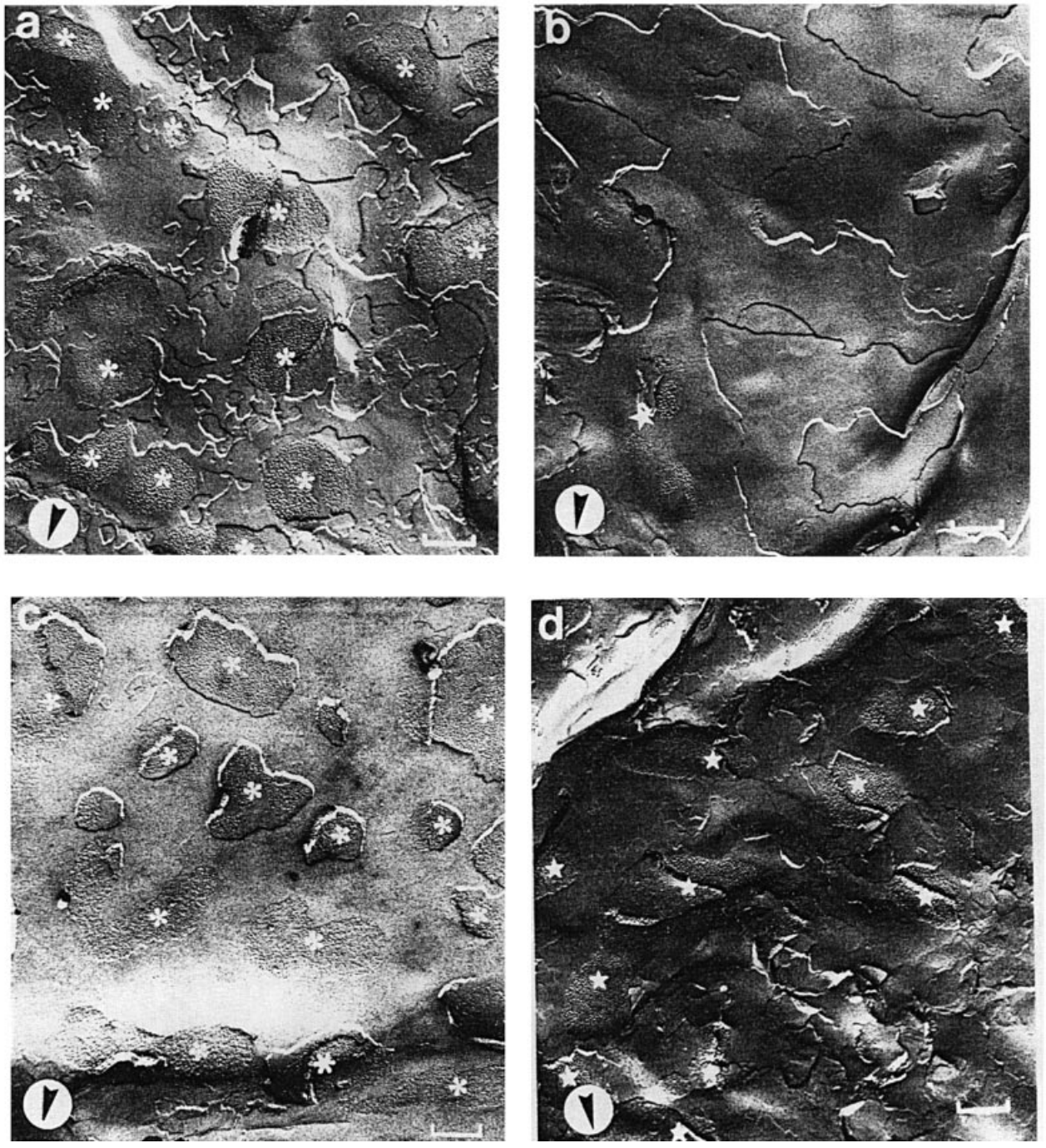

Figure 3. Freeze-fracture replicas show an increased number of corneodesmosomes on the surface of corneocytes from the outer SC of winter xerosis skin compared with those of normal skin. The morphology of intercellular spaces within the inner SC (*a*, *c*) and outer SC (*b*, *d*) from normal (*a*, *b*) and xerotic (*c*, *d*) skin strippings was analyzed on freeze-fracture electron micrographs. Corneodesmosomes appear as particle aggregates (asterisks and stars). *Arrowheads* indicate the direction of shadowing. *Scale bar*: 250 nm.

(**Fig 1*b***). As Dsg2 is only expressed in the basal layer of the epidermis and is not detected in the SC, these bands corresponded to entire Dsg1, and probably to two Dsg1 proteolytic fragments. As illustrated, the amount of immunodetected proteins varied from one individual (*lanes 1–3*) to another (*lanes 5–6*). For a given subject, the patterns obtained from the three sequential stripping extracts were identical (e.g., compare *lanes 1*, *2* and *3*).

When the same extracts were analyzed with G36-19 (**Fig 1*c***), with F28-27, or with both these MoAb (data not shown), which are directed against the central domain of Cdsn, proteolytic fragments of the protein were detected in all the extracts. They showed an apparent molecular mass ranging from 46 to 30 kDa. Various intensities in the immunoreactive peptides were observed. As above, the patterns obtained from the three successive stripping extracts of a given subject were identical.

When the extracts were analyzed with PG5.1, an antiplakoglobin MoAb (**Fig 1*d***), or with a mixture of PG5.1 and a MoAb raised against the carboxy-terminus of plakoglobin (data not shown), the entire 83 kDa plakoglobin and proteolytic fragments of the protein were detected in all the extracts tested. As above, the patterns obtained from the three sequential stripping extracts of a given subject were identical.

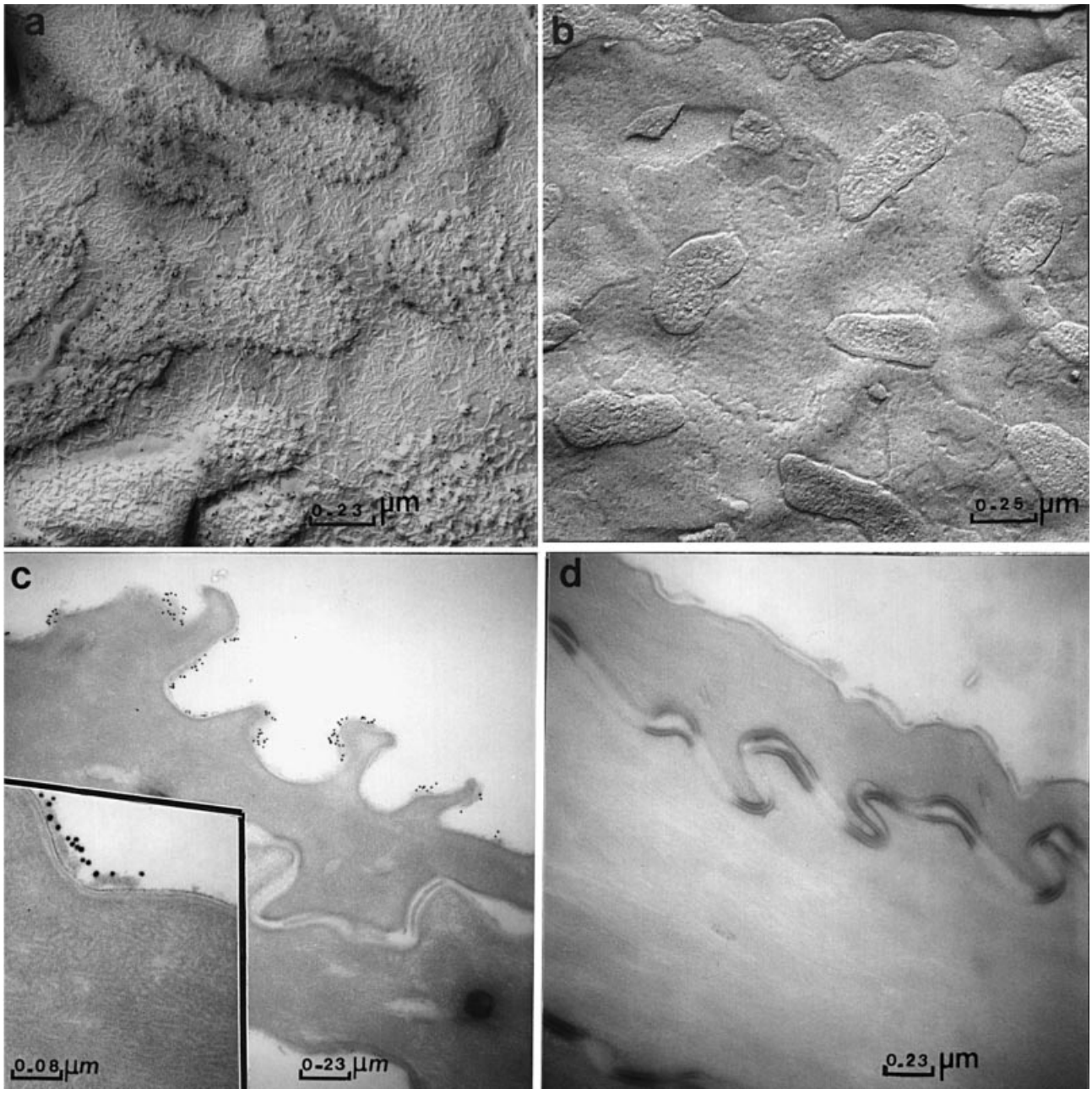

Figure 4. Freeze-fracture-labeled preparations of inner SC show Cdsn within the corneodesmosomes. Freeze-fractured samples of SC were immunogold labeled in the presence or absence of G36-19 MoAb. On platinum/carbon replicas of the fracture-labeled samples, G36-19 labeling is specifically detected on the particle aggregates morphologically identifiable as corneodesmosomes (*a*), whereas no labeling is observed in the absence of primary antibody (*b*). On electron micrographs of thin cross-sections of the same fracture-labeled samples, G36-19 labeling is specifically observed at the external surface of the samples in the core of corneodesmosomes (*c*), whereas no labeling is observed in the absence of primary antibody (*d*). The inset in (*c*) shows a higher magnification of a labeled corneodesmosome. *Scale bars* are shown on the micrographs.

When the extracts were analyzed with a cocktail of three antidesmoplakin MoAb, however, no immunoreactive bands were detected (data not shown).

The intensity of immunoblotting reactions and therefore the amounts of corneodesmosomal proteins (or of proteolysis fragments) detected seemed to be related to skin dryness. In particular, more entire Dsg1 and plakoglobin were usually observed in extracts of dry skin, suggesting incomplete proteolysis of corneodesmosomal components. Therefore, the immunoreactive bands were quantified by scanning and the reactivities were compared in two groups corresponding to the SC extracts of subjects with xerotic *versus* normal skin. A statistically significant increase in the quantified amount of the three corneodesmosomal proteins (or their proteolytic fragments) was observed in the first stripping extracts of xerotic *versus* normal skin (**Fig 2**). Similar results were obtained with the second stripping extracts (data not shown).

In the winter xerosis group as well as in the control group, the amounts of Dsg1 and plakoglobin were found to be strongly correlated ($r = 0.68$; $p < 10^{-6}$). In contrast, the amount of Cdsn fragments was found to be unrelated to either of these proteins ($r = 0.09$ with $p = 0.51$ and $r = 0.12$ with $p = 0.38$, respectively).

Persistence of nonperipheral corneodesmosomes in the upper SC of winter xerosis skin The SC of selected subjects, with either normal skin ($n = 3$) or skin affected by a severe winter xerosis ($n = 7$), was analyzed by freeze-fracture electron

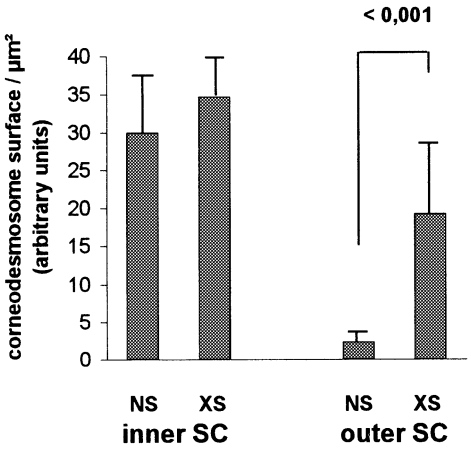

Figure 5. Quantification of corneodesmosome density at the surface of corneocytes confirms the reduced degradation of the structures in the outer SC of winter xerosis skin. The corneodesmosome density (area occupied by corneodesmosomes divided by total area of the micrograph) was measured on electron micrographs of the inner SC (two micrographs per individual) and the outer SC (four micrographs per individual) of normal (NS, $n = 2$) and winter xerosis (XS, $n = 3$) skin samples. Histograms representing the mean values show similar densities of corneodesmosomes in the outer SC of xerotic and normal skin. The density of corneodesmosomes in the upper SC of xerotic skin, however, was significantly increased compared with normal skin ($p < 0.001$). *Bars*, standard deviations.

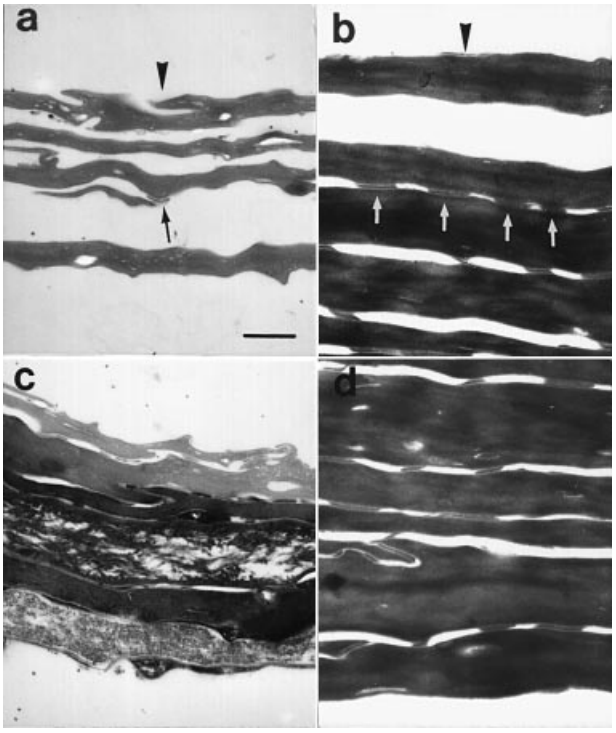

Figure 6. Conventional transmission electron microscopy confirms the persistence of corneodesmosomes in the outer SC of winter xerosis skin. Varnish-strippings of normal (*a*, *c*) and winter xerosis (*b*, *d*) skin were analyzed by conventional transmission electron microscopy. Note that, when combined, the micrographs of normal SC correspond to the whole sample. Only part of the total height of xerosis SC, which is much thicker, is shown, however. In the outer SC of normal skin (*a*), corneocytes are loose and corneodesmosomes (*arrows*) are scarcely observed. In the outer SC of xerotic skin (*b*), corneocytes are more cohesive and corneodesmosomes are numerous. In the inner SC of both normal (*c*) and xerotic (*d*) skin, corneodesmosomes are numerous. *Arrowheads* indicate the outer surface of the samples. *Scale bar*: 0.5 µm.

microscopy. This analysis confirmed, at the ultrastructural level, that corneodesmosome degradation is reduced in the SC of xerotic compared with normal skin. In the inner SC of both normal (**Fig 3*a***) and xerotic (**Fig 3*c***) skin, freeze-fracture replicas showed numerous structures, previously identified as corneodesmosomes (Elias *et al*, 1977; Boddé *et al*, 1990), appearing as particle aggregates of irregular shape all over the corneocyte surface. Moreover, the structures were shown to be specifically stained by anti-Cdsn antibody (**Fig 4**), confirming that they are corneodesmosomes. Most corneodesmosomes disappeared from the intercellular spaces in the outer SC of normal skin, except some that probably corresponded to the peripheral structures (**Fig 3*b***). In contrast to normal skin, however, in winter xerosis skin corneodesmosomes remained abundant over the whole corneocyte surface up to the desquamating cells (**Fig 3*d***). A quantitative analysis of the corneodesmosome density was performed (**Fig 5**). For normal skin, corneodesmosome density was found to be considerably lower in the outer than in the inner SC, as expected. Data obtained for the inner SC of normal and winter xerosis skin did not show statistical differences. In the outer SC of xerotic skin, however, the corneodesmosome density was significantly higher than in the outer SC of normal skin ($p < 0.001$).

Conventional transmission electron microscopy confirmed that the number of corneodesmosomes is increased and, consequently, that corneocytes are much more cohesive in the outer SC of xerotic skin, compared with the same layer of normal skin (**Fig 6**).

DISCUSSION

Although early models of SC cohesion ignored the role of corneodesmosomes, several recent studies have shown these adhesive structures to play an essential role, and their degradation at the skin surface to be the last event leading to desquamation. To characterize this degradation in normal skin and in winter xerosis skin, corneodesmosomes near the SC surface were analyzed using varnish stripping as a sampling procedure, and immunochemical and ultrastructural methods.

Winter xerosis is most probably a multifactorial disorder that may not be strictly induced by only cold temperature, although this is suggested by its name. For example various degrees of susceptibility to soap-induced skin dryness may also exist. This could explain the interindividual variations we observed in the same group, even though we took some precautions, e.g., the volunteers refrained from the use of moisturizers 2 d before the start of the study. A more restricted definition of the inclusion criteria, if it were possible, would probably reduce these variations.

Even if varnish stripping is a widely used method, several factors could influence the quantity of SC removed; these include the anatomic location, the degree of skin hydration, and the degree of corneocyte cohesiveness. Therefore, all strippings were performed at the same site on the leg. Moreover, the dry weight of removed material, as well as the amount of total proteins extracted, from normal and xerotic skin were analyzed and found to be similar. This shows that varnish penetrates the extracellular spaces of winter xerotic skin as well as normal skin, and validates the method for a comparative study.

When total proteins of the three sequential varnish-stripping extracts from both normal and winter xerosis skin were analyzed by western blotting with anti-Cdsn antibodies, the same complex pattern of proteolytic fragments with molecular masses of 46–40 and 36–30 kDa was revealed, in agreement with our previously published data (Guerrin *et al*, 1998). They most probably correspond to the 48–44 and 36–33 kDa components described by Lundström *et al* (1994). Lundström *et al* showed that tape-strippings of forearm SC allowed differences in the relative proportions of these forms, between deep and more superficial

[2]Simon M, Haftek M, Serre G: unpublished data.

corneocyte layers, to be observed. Here, we did not note such a difference in the sequential stripping extracts, probably because varnish stripping is a less discriminating method. Alternatively, this apparent discrepancy could be due to site heterogeneity.

The three sequential varnish-stripping extracts were also shown to contain the entire 160 kDa Dsg1 and/or Dsg1-derived fragments of 95 and 80 kDa. The 160 kDa Dsg1 was previously detected in plantar SC freed from loosely attached surface corneocytes, whereas 95 and 80 kDa fragments were detected in the partially desquamated surface corneocytes, collected by scraping (Lundström *et al*, 1994). These data suggest that Dsg1 is probably proteolyzed by the same protease(s), in plantar and nonpalmo-plantar SC, as previously proposed for Cdsn (Lundström *et al*, 1994). They also suggest that Dsg1 proteolysis already occurs in the deeper layers of nonpalmo-plantar SC, whereas it only takes place at the surface of plantar SC. Our results indicate that plakoglobin proteolysis may also occur in the deep SC. Whether this corresponds to the degradation of nonperipheral corneodesmosomes is unknown. In agreement with the latter possibility, degradation of corneodesmosomes is progressive, with the following sequence of events: partial vacuolation of the plug, encapsulation by lipid lamellae, and finally total degradation (Rawlings *et al*, 1994b; Fartasch, 1997; Haftek *et al*, 1998).

Entire Dsg1 and plakoglobin were observed in SC extracts from both normal and xerotic skin. The entire 52–56 kDa Cdsn was never detected in these extracts, however. This is probably explained by our recent observation showing the major Cdsn form in desmosomes to be the 46 kDa polypeptide. In fact, the first step in Cdsn processing by proteolysis, production of the 46 kDa form, seems to occur early after synthesis of the protein, even in the lamellar bodies.[2]

Extracellular (Cdsn) and transmembrane (Dsg1 and Dsc1) corneodesmosomal proteins as well as cytoplasmic desmosomal plaque proteins (plakoglobin) are all degraded in the upper SC. As previously suggested (Lundström *et al*, 1994), the enzyme(s) responsible for the degradation of Cdsn probably differ from that (those) involved in Dsg1 proteolysis, as the amounts of these proteins in the upper SC were not found to be statistically related. As a large amount of Cdsn and a small amount of Dsg1 were detected in some extracts, Cdsn does not appear to be an inhibitor of Dsg1 degradation, as we had previously suggested (Lundström *et al*, 1994). The amounts of Dsg1 and plakoglobin were found to be closely correlated, however, suggesting that their degradation is related and performed by the same protease(s). As plakoglobin is a cytoplasmic protein, this would indicate that at least the cytoplasmic domain of Dsg1 is cleaved. Accordingly, DG3.10, which recognizes an epitope in the carboxy-terminal tail of the cytoplasmic portion of Dsg1 located between amino acids 797 and 816 (Koch *et al*, 1990), detected a fragment of 80 kDa in the varnish-stripping extracts, i.e., smaller than the cytoplasmic domain of the protein.

The amount of corneodesmosomal proteins detected in the SC extracts was found to be significantly increased in xerotic *versus* normal skin. This is probably a consequence of reduced proteolysis and therefore reduced degradation of corneodesmosomes in winter xerosis, even if enhanced extraction of these proteins from xerotic skin cannot be totally excluded.

When the SC extracts were analyzed with antidesmoplakin MoAb, no signals were detected. As this negative result was obtained with a pool of three different antibodies, it is probably not the consequence of epitope masking but rather indicates either complete degradation of desmoplakin 1 and 2 to give nonimmunoreactive fragments in the lower layers of the SC or incorporation of the proteins into the cornified cell envelopes during cornification, as previously suggested (Steinert *et al*, 1998).

In the outer SC of severe winter xerosis skin, we detected an increased number of corneodesmosomes by conventional and freeze-fracture electron microscopy. This is in agreement with the western blotting analysis, which showed an increase of corneodesmosomal components in SC extracts of xerotic skin. As the number of corneodesmosomes is similar in the inner SC of normal and winter xerosis skin, the increased number of these adhesive structures at the SC surface of xerotic skin is necessarily the consequence of reduced degradation. The complete degradation of nonperipheral corneodesmosomes that normally occurs at the junction between the inner and outer SC is therefore absent or decreased in winter xerosis skin, as reported in soap-induced xerosis (Rawlings *et al*, 1994b). This defective proteolysis leads to the persistence of corneodesmosomes over the whole corneocyte surface that probably induces the defect in desquamation and the accumulation of scales observed in the xerotic skin. Persistence of nonperipheral corneodesmosomes in the outer SC is a characteristic of normal palmo-plantar skin (Skerrow *et al*, 1989; Chapman and Walsh, 1990; Serre *et al*, 1991). Such corneodesmosome retention has also been demonstrated in various ichthyoses and other hyperkeratotic diseases (Ghadially *et al*, 1992; Fartasch, 1997; Haftek *et al*, 1997). So, winter xerosis seems to be related to more severe hyperkeratotic skin diseases. The reasons for this reduced degradation of nonperipheral corneodesmosomes are unknown but it could be the consequence of skin dryness. For example, it could be related to the altered lipid composition observed in the SC of winter xerosis skin, as these lipids may control the activity of the proteases present in the extracellular spaces of the SC (Haftek *et al*, 1998; Sato *et al*, 1998), either directly or indirectly by microenvironment modulation. These enzymes may also be downregulated by the reduced water content of xerotic skin SC, as estimated by measuring skin surface conductance. Accordingly, both the environmental degree of humidity and glycerol, a well-known moisturizing agent efficient in xerosis treatment, have been shown to enhance *in vitro* corneodesmosomal degradation and corneocyte desquamation (Rawlings *et al*, 1995). Desquamation promoted by glycolic acid treatment was also recently proposed to be the consequence of an enhancement of corneodesmosomal degradation (Fartasch *et al*, 1997). Alternatively, constitutive inactivation of the SC proteases, due either to mutations of the enzymes or to a defect in their synthesis and maturation, as well as genetically determined resistance of corneodesmosomal components to proteolytic degradation, might lead to the persistence of such a high number of corneodesmosomes at the surface of xerotic skin corneocytes. Whatever its cause, retention of nonperipheral corneodesmosomes seems to be directly responsible for hyperkeratosis and abnormal desquamation of winter xerosis.

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